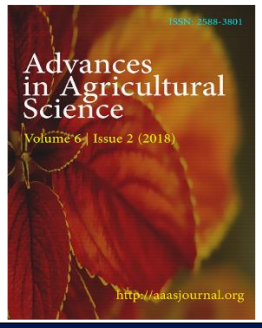
Advances in Agricultural Science

Volume 6 (2018), Issue 02, 50-58

Journal homepage: www.aaasjournal.org

ISSN: 2588-3801



Research Article Open Access

Potential role of some biofertilizers, plant nutrients and a biocide for the management of reniform nematode, *Rotylenchulus reniformis* infecting sunflower in Egypt

Ahmed El-Sayed Ismail [1*]

[1] *Department of Plant Pathology, National Research Center, Dokki, 12622, Giza, Egypt.*

ARTICLE INFO

Article history:
Received: January 18, 2018
Revised: February 20, 2018
Accepted: March 12, 2018
Available online: May 14, 2018

Keywords:
Biocides
Nematode biocontrol
Rotylenchulus reniformis
Plant nutrients
Sunflower

[*] Corresponding Author;
E. Mail: iismail2002@yahoo.co.uk

ABSTRACT

The reniform nematode, *Rotylenchulus reniformis* attacks a wide range of crops including sunflower, *Helianthus annuus* in Egypt as well as in many parts of the world. Elimination of the nematodes has received attention to minimize damage to plants. Thus, the present study aims to estimate the probable effects of three rates of some biofertilizers, plant nutrients and a biocide on the development of *R. reniformis* in sunflower and growth of the plant. Three Egyptian of bio-fertilizers (BF), i.e. Nitrobien (at doses 0.034, 0.068 and 0.136), Rizobactrein (at doses 0.017, 0.034 and 0.068) and Blue-green (at doses 0.2, 0.4 and 0.8); and three Egyptian plant nutrients (PN) i.e. Citrein (at doses 0.1, 0.2 and 0.4), Kotangein (at doses 0.01, 0.02 and 0.03) and Kapronite (at doses 1.0, 2.0 and 4.0) as well as the biocide Nemaless (at doses 0.005, 0.01 and 0.02) were evaluated as a lower rate, the recommend rate and a higher rate; respectively for control of *R. reniformis* and improvement of sunflower cv. Giza 101 under greenhouse conditions 30 ± 5 ºC. All the evaluated compounds significantly reduced (P≤ 0.05 and/ or 0.01) the number of juveniles in soil, swollen females and egg-laying females on roots. The reduction varied greatly according to the type of experimented products and rate of application. The highest reduction in the nematode populations, swollen females and egg-laying females was attained with seed coating by Rizobactrein followed by Nitrobien as bio-fertilizers while, the least reductions were obtained by using Blue-green as alga biofertilizer followed by Nemaless as a biocide. Application of the plant nutrients, Kotangein as seed coating and Kapronite as soil amendment were effectively decreased the development of the nematode stages. Citrein as a foliar spray nutrient was the least effective. Generally, Rizobactrein and Nitrobien as biofertilizers; Kapronite and Kotangein as plant nutrients proved to be the most effective for controlling *R. reniformis* and gave the greatest growth of sunflower plants as compared with the rest treatments.

Introduction

Over-reliance on the use of synthetic pesticides in crop protection has resulted in disturbances to the environment, pest resurgence, pest resistance to pesticides and lethal and sub-lethal effects on non-target organisms, including humans (Prakash and Rao, 1996). These side effects have raised public concern about the routine use and safety of chemical nematicides. Also, increases in the populations of plant-parasitic nematodes and a tendency to use ever

greater quantities of pesticides are causing ever greater environmental problems, that is, provision of sufficient clean food whilst at the same time protecting water supplies and wild life habitats. In Egypt, plant parasitic nematodes, especially reniform nematode, *Rotylenchulus reniformis* are important pests and cause considerable loss to many economic crops including sunflower (Johnson and Fassuliutis, 1984; Oteifa, 1987).

Therefore, management of this nematode has received attention to reduce damage by encourage scientists to search for synthetic pesticides alternatives. (El-Gindi *et al.*, 2005a) and (Ismail and Hasabo, 2000) found that Nemaless (a water suspension of *Serratia marcescens* Bizio containing 1×10^9 bacterium cells / ml water, produced by the Egyptian Ministry of Agriculture and Land Reclamation) reduced the different stages of *Meloidogyne incognita* . Also, these bacterium cells used as potential biocontrol agents against different parasitic nematodes (Mercer *et al.*, 1992; Abd-Elgawad and Mohamed, 2006; Kassab S.A. *et al.*, 2017). Also, use of selected marine algae as biocidal agents offers a potential approach to suppress the nematode pests of agricultural crops (Paracer *et al.*, 1987; Ismail and Hasabo, 2000; Youssef and Eissa, 2014). The interrelationships between nematode populations, nutrient elements and plant growth have been reported (Kirkpatrick *et al.*, 1964; Ismail and Hasabo, 2000). Using of powdered sulphur on soil of garlic field caused significant reductions in *Tylenchorhynchus* spp. and *R. reniformis* populations (Kassab and Hafez, 1990).

With respect to the relation between trace elements and nematode populations and pathoginicity studies, many reports have been done. (Vangundy and Martin, 1961) reported that copper in the leaves of sweet orange seedlings was reduced by *Tylenchulus semipenetrans*. (Ashoub A. A., 1978) found that eggplants provided with iron (Fe) nutrient solution harboured less number of *R. reniformis* than that of non-treated plants as well as, *R. reniformis* was less in cases of Fe or Zn deficiency but not with Mo – deficiency (Aboul-Eid *et al.*, 1980). Also, (El-Gindi *et al.*, 2005b) found no significant differences between nitrobien and phosphorine as biofertilizers in reducing *M. incognita* population and cowpea growth responses, however, nitrobien seems to be more effective than phosphorine on the above mentioned parameters.

Thus, the objectives of the present study aims to estimate the probable effects of some biofertilizers, plant nutrients and a biocide on the development of *R. reniformis* in sunflower, *Helianthus annuus* as well as growth of the plant in Egypt.

Materials and Methods

Two Egyptian biofertilizers i.e. Nitrobien (at doses 0.034, 0.068 and 0.136) and Rizobactrein (at doses 0.017, 0.034 and 0.068) containing the nitrogen fixing bacteria and one plant nutrient such as Kotangein (at doses 0.01, 0.02 and 0.03) containing a mixture of microelements (Fe, Zn and Mn) with sulphur were used as seed coating to sunflower seeds. Other treatments included foliar application with one plant nutrient i.e. Sitrein (mixture of 6% Fe, Mn and Zn with 15% citric acid) was used at doses 0.1, 0.2 and 0.4. Blue- green algae as biofertilizer was used at doses 0.2, 0.4 and 0.8; Kapronite (at doses 1.0, 2.0 and 4.0) was used as plant nutrient containing a mixture of elemental sulphur and substances enriched with P, K, Ca, Mg and Nemaless (at doses 0.005, 0.01 and 0.02) was applied as a biocide containing a strain of bacteria *Serratia marcessens* were used as soil treatment. All the previous biofertilizers, plant nutrients and a biocide were applied at the recommended rate as well as half and double rates.

Seeds of sunflower cv. Giza 101 were sown in 20 cm diameter clay pots filled with 2 kg autoclaved soil mixture of sand and clay (1:1, v:v). After 15 days of germination, only one healthy plant was kept in each pot and six replicates were prepared from each treatment and each plant was inoculated with 1000 freshly hatched *Rotylenchulus reniformis* fourth stage juveniles (J_4). Untreated pots inoculated

with nematodes served as control. Pots were arranged in a randomized complete block design in a greenhouse at 30± 5ºC. Soil treatments were applied at sowing time; foliar spray nutrients were applied twice (at 3 weeks old plants then 3 weeks later). Seed coating treatments were made by mixing the seeds in Arab gum and the tested material for 2-5 minutes and then left to dry for 4 hours before sowing. Seventy days after nematode inoculation, sunflower plants were gently uprooted and the fourth- stage juveniles (J_4) in the soil were extracted by sieving and centrifugation (Barker *et al.*, 1986). Number of swollen females and egg-laying females were counted for the whole root system. Lengths, fresh and dry weights of both shoot and root systems were recorded. The percentages reduction or increase in the nematode population or plant growth parameters as compared to untreated plants were calculated.

Data analysis

In both experiments during two successive seasons 2015 and 2016, obtained data on sunflower growth components were collected. Data were also collected on number of fourth stage of juveniles (J_4) in soil, each of swollen females and egg-laying females from all the treatments. All data were pooled together and means were compared statistically using the Fisher, s Least Significant Difference (L.S.D.).

Results

Tables 1 and 2 showed that using of the biofertilizers (BF), the plant nutrients (PN) and the biocide at the three rates significantly ($P \leq 0.05$ and / or 0.01 levels) reduced numbers of juveniles in soil, swollen females and egg-laying females on roots as compared to untreated plants. In the previous nematode stages, statistical differences at 0.05 and / or 0.01 levels were noted within some treatments. The reduction greatly varied according to the type of the evaluated materials. So, the highest decrease in numbers of juveniles in soil, swollen females and egg-laying females was more clearer with seed coating by Rizobactrein followed by Nitrobien biofertilizers (Table 1). However, the least decreases in the nematode stages were obtained when plants treated with Blue green algae biofertilizer followed by Nemaless biocide. With respect to the impact of the tested plant and soil nutrients (Table 2), application of Kotangein as seed coating and Kapronite as soil amendment showed highest percentage reduction; respectively in the numbers of juveniles in soil, swollen females and egg-laying females followed by Citrein treatment.

The influence of the various rates of BF, PN and Nemaless on the growth of sunflower plants infected with *R. reniformis* is presented in Tables 3 and 4. All treatments with the different rates significantly increased plant growth ($P \leq 0.05$ and / or 0.01 levels), with some exceptions, as compared to untreated plants. Statistical differences at 0.05 and / or 0.01 levels in all shoot and root systems growth parameters were observed within some treatments. However, insignificant variations, with some exceptions, were noted between the tested three rates of each treatment in all plant growth criteria (Tables 3 and 4). The increase in plant growth parameters varied according to the type of the evaluated materials. Therfore, the highest increase in lengths, fresh and dry weights of both shoot and root systems were obtained by using Rizobactrein followed by Nitrobien as biofertilizers (Table 3). Using of Kotangein (seed coating) and Kapronite (soil amendment) as plant nutrients showed the highest percentage increase in the plant growth parameters while; the least increases were observed in plants treated with Citrein (Table 4). Clearly, stronger responses were obtained in shoot growth parameters compared to root growth parameters (Tables 3 and 4).

Discussion

The evaluated biofertilizers, plant nutrients and a biocide had a negative effect on the development of

Table 1. Effect of some bio-fertilizers and a biocide on the development of *Rotylenchulus reniformis*.* (Mean of two successive seasons, 2015 and 2016).

Treatment and type of application	Dose	No. of juveniles in soil	Reduction%**	No. of swollen females / root	Reduction%	No. of egg-laying females / root	Reduction%
Nitrogenous bio-fertilizers (gm / gm of seeds)							
Nitrobien	0.034	2150	50.9	160	36.0	110	24.1
(seed coating)	0.068	1900	56.6	145	42.0	95	34.5
	0.136	1370	68.7	133	46.8	81	44.1
Rizobactrein	0.017	1395	68.2	110	56.0	88	39.3
(seed coating)	0.034	1100	74.9	98	60.8	75	48.3
	0.068	630	85.6	78	68.8	51	64.8
Blue-green Algae (gm / pot)							
Blue – green	0.2	3900	11.0	233	6.8	140	3.5
(soil)	0.4	3760	14.2	215	14.0	131	9.7
	0.8	3250	25.8	210	16.0	129	11.0
Biocide (ml / pot)							
Nemaless	0.005	3050	30.4	212	15.2	128	11.7
(soil)	0.01	2860	34.7	190	24.0	110	24.1
	0.02	2410	45.0	177	29.2	107	26.2
Control	-	4380	-	250	-	145	-
L.S.D. 5%	-	410	-	15	-	17	-
L.S.D. 1%	-	620	-	23	-	31	-

*Data given represent the mean of six replicates.

**Percentage reduction compared to control.

R. reniformis on sunflower. This was evident by the lower numbers of juveniles in soil, lower numbers of swollen females and egg-laying females and this coincided with improvement in plant growth of the treated pots. These findings are in harmony with those of (Featonby-Smith and Van Staden, 1983) (Kassab and Hafez, 1990) (Ali and Kamal, 1998) (Abd-Elgawad and Mohamed, 2006; Kassab S.A. *et al.*, 2017). They stated that the application of iron nutrient solution; brown alga; powdered sulphur and diluted liquid culture of the bacteria *Serratia marcessens* significantly suppressed several species of plant-parasitic nematodes and improved the growth of the host crops. Also, (El-Sherif *et al.*, 1994) (Ali, 1996; Ismail and Hasabo, 2000; Abd-Elgawad and Mohamed, 2006; Kassab S.A. *et al.*,

Table 2. Effect of some plant and soil nutrients on the development of *Rotylenchulus reniformis*.* (Mean of two successive seasons, 2015 and 2016).

Treatment and type of application	Dose	No. of juveniles in soil	Reduction%**	No. of swollen females / root	Reduction%	No. of egg-laying females / root	Reduction%
Foliar spray nutrients (ml / plant)							
Citrein	0.1	3430	21.7	215	14.0	136	6.2
	0.2	2950	32.7	195	22.0	125	13.8
	0.4	2710	38.1	183	26.8	110	24.1
Seed coating (gm / gm of seeds)							
Kotangein	0.01	1210	72.4	153	38.8	110	24.1
	0.02	950	78.3	110	56.0	93	35.9
	0.03	910	79.2	98	60.8	78	46.2
Soil amendment (gm / pot)							
Kapronite	1.0	2100	52.1	198	20.8	122	15.9
	2.0	1830	58.2	173	30.8	107	26.2
	4.0	1100	74.9	161	35.6	98	32.4
Control	-	4380	-	250	-	145	-
L.S.D. 5%	-	1010	-	12	-	14	-
L.S.D. 1%	-	1400	-	19	-	25	-

*Data given represent the mean of six replicates.

**Percentage reduction compared to control.

2017) reported that liquid cultures of *Serratia* sp. (the major component of the biocide Nemaless) or its filtrates inhibited egg hatching and juvenile survival of different plant parasitic nematodes. The role of these bacteria may be attributed to the accumulation of toxic metabolites of these bioagents in soil. These metabolites may have a direct lethal effect on nematodes (Dicklow *et al.*, 1993), or have some physiological and / or behavioral effects such as disorder of neuromuscular junctions or through suppression of hatching, movement, feeding and invasion to host tissue (Mishra *et al.*, 1987; Kluepfel *et al.*, 1993). For addition, ammonia produced by modifying bacteria during natural decomposition of nitrogenous products has been often implicated in the control of plant parasitic nematodes (Rodriguez-Kabana, 1986). Fatty acids, volatile compounds, hydrogen sulfide, enzymes, hormones, alcohol and phenolic compounds are among the bacterial metabolic products implicated in the management of plant parasitic nematodes (Mishra *et al.*, 1987; Ismail and Hasabo, 2000; Abd-Elgawad and Mohamed, 2006; Kassab S.A. *et al.*, 2017). These products may be toxic to nematodes directly or it may indirectly suppress nematode population by modifying the rhizosphere environment.

Table 3. Effect of some bio-fertilizers and a biocide on the growth of sunflower infected with *R. reniformis*.* (Mean of two successive seasons, 2015 and 2016).

Treatment and type of application	Dose	Shoot growth						Root growth					
		Fresh weight (g)	Increase % **	Dry weight (g)	Increase %	Length (cm)	Increase %	Fresh weight	Increase %	Dry weight (g)	Increase %	Length (cm)	Increase %
Nitrogenous bio-fertilizers (gm / gm of seeds)													
Nitrobien	0.034	7.5	36.4	1.8	100	45.1	17.8	7.6	182	2.0	186	22.6	28.4
(seed coating)	0.068	9.0	63.6	2.3	156	47.3	23.5	8.3	207	2.4	243	24.3	38.1
	0.136	12.0	118.2	3.1	244	48.9	27.7	9.7	259	2.9	314	26.2	48.9
Rizobactrein	0.017	12.0	118.2	2.2	144	46.5	21.4	8.1	200	2.1	200	25.3	43.8
(seed coating)	0.034	13.9	152.7	2.7	200	49.8	30.0	9.3	244	2.6	271	26.1	48.3
	0.068	14.9	170.9	3.9	333	51.3	33.9	11	311	3.1	343	28.3	60.8
Blue-green Algae (gm / pot)													
Blue – green	0.2	6.0	9.1	1.5	66.7	43.6	13.8	6.5	141	1.5	114	17.8	1.1
(soil)	0.4	7.9	43.6	1.9	111	45.9	19.8	7.1	163	1.9	171	20.1	14.2
	0.8	9.3	69.1	2.4	167	48.4	26.4	8.2	204	2.1	200	21.7	23.3
Biocide (ml / pot)													
Nemaless	0.005	6.5	18.2	1.6	77.8	44.8	17.0	7.1	163	1.9	171	19.7	11.9
(soil)	0.01	8.6	56.4	2.1	133	46.7	21.9	7.9	193	2.2	214	21.4	21.6
	0.02	9.9	80.1	2.8	211	49.1	28.2	8.8	226	2.6	271	23.5	33.5
Control	-	5.5	-	0.9	-	38.3	-	2.7	-	0.7	-	17.6	-
L.S.D. 5%	-	3.6	-	0.7	-	6.3	-	4.4	-	0.4	-	4.6	-
L.S.D. 1%	-	5.3	-	1.8	-	8.6	-	6.1	-	0.6	-	6.2	-

*Data given represent the mean of six replicates.

**Percentage increase compared to control.

The increase in plant growth as compared to the untreated control could be attributed to decreasing of the nematode population and to the addition of organic or inorganic compounds contained in the bacterial metabolites. Moreover, most of these products improved the physical and chemical properties of soil and provides the soil with components that help solubilization and absorption of many macro and micro elements by plants which encourage plant growth. The positive benefits from seed coating with Kotangein which contained a mixture of microelements (Fe, Zn and Mn) with sulphur have been attributed increased root uptake capacity because of enhanced root development and hair formation in response to secretion of plant

Table 4. Effect of some plant and soil nutrients on the growth of sunflower infected with *R. reniformis*.* (Mean of two successive seasons, 2015 and 2016).

Treatment and type of application	Dose	Shoot growth						Root growth					
		Fresh weight (g)	Increase % **	Dry weight (g)	Increase %	Length (cm)	Increase %	Fresh weight	Increase %	Dry weight (g)	Increase %	Length (cm)	Increase %
Foliar spray nutrients (ml / plant)													
Citrein	0.1	9.1	65.5	2.4	167	43.9	15	3.6	33	1.3	86	22.8	29.6
	0.2	10.1	83.6	3.6	300	44.3	16	4.1	52	1.4	100	23.3	32.4
	0.4	10.6	92.7	3.7	311	45.6	19	4.9	82	1.8	157	24.6	39.8
Seed coating (gm / gm of seeds)													
Kotangein	0.01	10.8	96.3	3.1	244	47.6	24	4.9	82	1.5	114	24.7	40.3
	0.02	11.3	106	3.9	333	48.1	26	5.3	96	1.8	157	25.2	43.2
	0.03	11.9	116	4.0	344	50.3	31	5.9	119	2.1	200	25.9	47.2
Soil amendment (gm / pot)													
Kapronite	1.0	9.9	80.0	2.8	211	45.1	18	4.1	52	1.2	71	23.6	34.1
	2.0	10.2	85.5	3.7	311	46.7	22	5.1	89	1.7	143	24.1	36.9
	4.0	10.9	98.2	3.9	333	48.4	26	5.3	96	1.8	157	25.3	43.8
Control	-	5.5	-	0.9	-	38.3	-	2.7	-	0.7	-	17.6	-
L.S.D. 5%	-	2.9	-	0.6	-	6.3	-	0.9	-	0.4	-	3.2	-
L.S.D. 1%	-	4.1	-	1.7	-	8.2	-	1.1	-	0.6	-	4.6	-

*Data given represent the mean of six replicates.

**Percentage increase compared to control.

growth hormones (Owens and Novotny, 1960; Ismail and Hasabo, 2000).

Conclusion

Overall, it could be concluded that the net effect of these substances is therefore, combating *R. reniformis* populations and improving sunflower growth through a non-toxic, biological control system which is clearly evident in this study.

More studies are, however, in progress to elucidate the action of afore-mention products on other plant-parasitic nematodes attacking various economic crops.

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